An ant-plant mutualism induces shifts in the protist community structure of a tank-bromeliad

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Abstract

Although ants may induce community-wide effects via changes in physical habitats in terrestrial environments, their influence on aquatic communities living in plant-held waters remains largely underexplored. The neotropical tank-bromeliad *Aechmea mertensii* (Bromeliaceae) occurs along forest edges in ant-gardens initiated by *Camponotus femoratus* or by *Pachycondyla goeldii*. Its leaves form wells that hold rainwater and provide suitable habitats for many aquatic organisms. We postulated that these ant–plant mutualisms indirectly affect the microbial community structure via changes in the environmental conditions experienced by the plants. To test this hypothesis, we analyzed the protist communities from 63 tank-bromeliads associated with either *C. femoratus* or *P. goeldii* (hereafter Cf-*Aechmea* and Pg-*Aechmea*) along a forest edge in French Guiana. For each plant, a large number of environmental variables (including habitat structure, food resources, incident radiation and the presence of aquatic invertebrates) were quantified to determine their relative importance in driving any observed differences across ant-associated plants. Pg-*Aechmea* are located in sun-exposed areas and hold low volumes of water and low amounts of detritus, whereas Cf-*Aechmea* are located in partially shaded areas and accumulate higher amounts of water and detritus. Protists (i.e., protozoa and algae) inhabiting Cf-*Aechmea* exhibit greater richness and abundances than those in Pg-*Aechmea*. Variations in detritus content, number of leaves, incident radiation, and the epiphyte richness of the ant-garden were the main factors explaining the variation in protist richness. A shift in the functional group composition of protists between bromeliads tended by different ant species suggested that mutualistic ants indirectly mediate changes in the microbial food web.

Zusammenfassung

Obgleich Ameisen gemeinschaftsübergreifende Effekte auslösen können, indem sie in terrestrischen Lebensräumen Änderungen des physikalischen Habitats bewirken, ist ihr Einfluss auf aquatische Gemeinschaften von Phytotelmata weitgehend

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unerforscht. Die neotropische Trichterbromelie *Aechmea mertensii* (Bromeliaceae) kommt entlang von Waldrändern in Ameisengärten, die von *Camponotus femoratus* bzw. *Pachycondyla goeldii* angelegt werden, vor. Ihre Blätter bilden Trichter, die Regenwasser auffangen und ein Habitat für zahlreiche aquatische Organismen sind.

Wir postulierten, dass die mutualistischen Beziehungen zwischen der Pflanze und den Ameisenarten indirekt die Gemeinschaftsstruktur der Mikroorganismen beeinflussen - und zwar durch Änderungen der Umweltbedingungen, denen die Pflanzen ausgesetzt sind. Um diese Hypothese zu testen, analysierten wir entlang eines Waldrandes in Französisch-Guayana die Einzellergemeinschaften von 63 Trichterbromelien, die mit *C. femoratus* bzw. *P. goeldii* assoziiert waren (im Folgenden: Cf-*Aechmea* bzw. Pg-*Aechmea*). Für jede Pflanze wurde eine große Zahl von Umweltfaktoren (Habitatstruktur, Nahrungsressourcen, Strahlungsintensität, aquatische Wirbellose) untersucht, um deren relative Bedeutung für Unterschiede zwischen den mit Ameisen assoziierten Pflanzen zu bestimmen. Pg-*Aechmea* befinden sich an sonnenexponierten Stellen und enthalten geringe Mengen Wasser und Detritus, während Cf-*Aechmea* in partiell beschatteten Bereichen vorkommen und größere Mengen Wasser und Detritus speichern. Die Einzeller (d.h., Protozoen und Algen) von Cf-*Aechmea* zeigten größeren Formenreichtum und höhere Abundanzen als die in Pg-*Aechmea*. Detritusgehalt, Anzahl der Blätter, Einfallstrahlung und der Artenreichtum der Epiphyten im Ameisengarten waren die wichtigsten Erklärungsfaktoren für die Variation im Formenreichtum der Einzeller. Eine Verschiebung in der Zusammensetzung der funktionellen Gruppen der Einzeller zwischen Bromelien, die von verschiedenen Ameisenarten betreut wurden, legt nahe, dass die mutualistischen Ameisen indirekt Änderungen im mikrobiellen Nahrungsnetz vermitteln.

Keywords: *Aechmea mertensii*; Ant-garden; Protists; Biodiversity; *Camponotus femoratus*; *Pachycondyla goeldii*; Phytotelmata; French Guiana

Introduction

Ants are involved in an interlocking set of mutualisms (McKey et al. 2005) and ant-gardens are recognized as belonging to the most sophisticated of all symbioses between ants and flowering plants in lowland Amazonia (Orivel & Leroy 2011). Ant-gardens are initiated by a few species of ants whose founding queens and/or workers build arboreal carton nests. Ants collect and incorporate into the walls of their nests the seeds of selected epiphyte species which then germinate and grow (Orivel & Dejean 1999), so that the plant roots stabilize and anchor the entire structure in the supporting tree. The seeds of these epiphytes attract ants through nutritional rewards called elaiosomes, but even in their absence these seeds remain attractive to ant-garden-building ants thanks to compounds on the seed cuticle (Orivel & Dejean 1999; Youngsteadt, Nojima, Haberlein, Schulz, & Schal 2008). In turn, the plants benefit from seed dispersal and protection from defoliating insects (Orivel & Leroy 2011). Among the Neotropical epiphytes, the tank-bromeliad *Aechmea mertensii* Schult.f. (Bromeliaceae) only occurs (over its full range) in association with ant-gardens (Benzing 2000). In French Guiana, *A. mertensii* is found in pioneer growths in ant-gardens initiated either by *Camponotus femoratus* Fabr. or by *Pachycondyla goeldii* Forel (Corbara & Dejean 1996). The leaves of *A. mertensii* are tightly interlocking, forming several wells that collect water and organic detritus. These wells are characteristic of tank-forming bromeliads acting as phytotelmata. The aquatic communities inhabiting tank-bromeliads consist of micro- and macroinvertebrates, occasionally vertebrates (i.e., anurans) (Kitching 2000; Frank & Lounibos 2009), and diverse microorganisms (Brouard et al. 2012). Since *A. mertensii* is both a phytotelm- and an ant-garden bromeliad, it was deemed a relevant model for studying the role of interspecific mutualistic interactions in shaping aquatic communities in nature (Céréghino, Leroy, Dejean, & Corbara 2010; Céréghino et al. 2011).

We investigated the effect of these ant–plant mutualisms on aquatic microorganisms by analyzing protist (protozoa and algae) communities from 63 bromeliads associated with either *C. femoratus* or *P. goeldii*. In aquatic environments, protozoa control bacterial prey and release large amounts of dissolved inorganic nutrients (Sherr & Sherr 2002), while algae are frequently dominant primary producers and have recently been identified as a ubiquitous and relevant component of the aquatic communities of tank-bromeliads (Brouard et al. 2011, 2012). A previous study has shown that, as dispersal agents for *A. mertensii*, *P. goeldii* and *C. femoratus* indirectly influence the physical characteristics of the plant by determining the location of the seedling in areas ranging from sun-exposed to partially shaded (Leroy, Corbara, Dejean, & Céréghino 2009). We thus hypothesized that the density of algae would increase with the plant's exposure (i.e., from shaded to exposed areas), and thus would be more relevant to the microbial food web in Pg-*Aechmea* than in Cf-*Aechmea*. The latter constitute more complex and larger habitats and receive larger amounts of leaf litter from overhanging trees. Therefore, we expected larger densities and a greater richness of protists in Cf-*Aechmea* compared to Pg-*Aechmea*. Finally, we projected that ants would indirectly influence the microbial food web due to changes in the numerical importance of the functional groups of protists.

Materials and methods

Study site and ant-gardens

This study was conducted in French Guiana in March 2009 in a secondary forest located along an 11-km-long dirt road near the Petit-Saut Dam (latitude: 5°03′43″N; longitude: 53°02′46″W; elevation a.s.l.: 80 m). The climate is tropical moist, with 3000 mm of yearly precipitation distributed over 280 d. There is a major drop in rainfall between September and November (dry season) and another shorter, more irregular dry period in March. The maximum monthly temperature averages 33.5 °C, and the monthly minimum is 20.3 °C.

All samples were taken from *A. mertensii* bromeliads rooted on well developed ant-gardens inhabited either by *C. femoratus* and *Crematogaster levior* ($n = 31$) or by *P. goeldii* ($n = 32$). *A. mertensii* shifts from a funnel-like, crateriform shape (Cf-*Aechmea*) to a bulbous, amphora shape (Pg-*Aechmea*) along a gradient from partially shaded to sun-exposed locations within the same area of forest edge. *C. femoratus* is a polygynous arboreal formicine species living in a parabiotic association with the myrmicine species *Cr. levior*; that is to say, they share the same nests and trails but shelter in different cavities of the nests (Orivel & Leroy 2011). Their large, polydomous colonies and aggressiveness identify them as territorially dominant arboreal species in Neotropical rainforest canopies. Conversely, *P. goeldii* is a monogynous (single queen) arboreal ponerine species with comparatively smaller populations, although the colonies may be polydomous (Corbara & Dejean 1996; Dejean et al. 2000).

Habitat variables and sampling

To avoid the influence of ontogenetic gradients, all sampled bromeliads were at the flowering stage of the plant life cycle. For each tank-bromeliad, we recorded fifteen variables. Plant height (cm) was measured as the distance from the bottom of the body to the top of the crown. Plant width (cm) was the maximum distance between the tips of the leaves (mean of two measurements at 90°). After recording the total number of leaves and number of distinct wells constituting the tank (see Appendix A: Figure 1), the leaf display was estimated as the proportion of horizontal and vertical leaves (%). The length and width of the longest leaf were also recorded, as well as the height and diameter (two random measurements at 90°) of the reservoir (cm). This first set of ten variables described the vegetative traits of the bromeliads. We then recorded the elevation above the ground (m) and the number of epiphyte species (including *A. mertensii*) rooted on the ant-garden. The percentages of total incident radiation above the bromeliads were calculated using hemispherical photographs, GPS locations, and an image processing software (Gap Light Analyzer 2.0; Frazer, Canham, & Lertzman 1999), as described in Leroy et al. (2009). This set of three variables described the local conditions of the bromeliads in the supporting ant-gardens. The water volume extracted (mL) and the amount of fine particulate organic matter (FPOM; 1000–0.45 μm in size) were recorded. These two variables were chosen to describe the amount of water available to freshwater organisms and the amount of food resources at the base of the food webs.

To sample the water retained in *A. mertensii*, we carefully emptied the wells (4–13 wells per plant) using 5-mL and 10-mL micropipettes with the end trimmed to widen the orifice. This technique allowed us to collect most of the water (Jocqué, Kernahan, Nobes, Willians, & Field 2010). We then recorded the volume extracted from each bromeliad as well as the amount of FPOM, which was expressed as preserved volume (mm^3 after decantation in graduated test-tubes; see also Harlan & Paradise 2006). A subsample of 20% of the collected volume was fixed with 2% (v/v) glutaraldehyde to count the protists and other microorganisms. The remaining water (and its contents) was preserved in the field in 4% formalin (final concentration) to count the aquatic invertebrates (see below).

Protist diversity

Subsamples (1–5 mL) for counting algae and heterotrophic nanoflagellates (hereafter, HNF) were stained with primulin, collected onto 0.8 μm pore-size black Nuclepore filters, mounted with oil between a glass slide and a cover slip, and then stored at −20 °C. The slides were examined under UV light (340–380 nm) at 1100× magnification using a Leica DC 300F epifluorescence microscope. Pigmented protists were distinguished by detecting the red autofluorescence of chlorophyll *a* under blue light (450–490 nm) excitation.

Subsamples of 2 mL were placed into plankton chambers to count ciliates and naked or testate amoebae. Counting was conducted using an inverted Leica microscope at 400× magnification by scanning the entire chamber area. Protists were identified to morphospecies based on morphological characteristics (i.e., size, cell shape, pigmentation, flagella, cilia, shell), and the abundance of each species in different functional groups (i.e., algae: phototrophs; HNF: raptorial feeders of small bacteria; ciliates: filter feeders of suspended bacteria; naked and testate amoebae: consumers of bacteria, algae, and non-living organic material using pseudopodia) was estimated for each sampled plant. Protist diversity was expressed as species richness and through the Shannon index. Species richness (S) was determined as the number of protist morphospecies in each bromeliad. The Shannon index (H') was calculated as:

$$H' = -\sum p_i \ln p_i$$

where p_i is the relative biomass of species i and is calculated as the ratio between the individual biovolume of a species and the biovolume of the complete protist community in each sample.

Table 1. Differences in habitat characteristics and protist variables between *Aechmea mertensii* associated with *Camponotus femoratus* (Cf-*Aechmea*) and those associated with *Pachycondyla goeldii* (Pg-*Aechmea*). IR: incident radiation, WV: water volume, FPOM: fine particulate organic matter, NW: number of wells, and HNF: heterotrophic nanoflagellates.

	Cf-*Aechmea*		Pg-*Aechmea*		ANOVA		
	Mean ± SE	Range	Mean ± SE	Range	df	*F*	*P*
Habitat characteristics							
IR (%)	25.1 ± 9.9	12.5–48.2	53.1 ± 19.5	22.3–86.1	62	58.8	<0.001
WV (mL)	129.0 ± 89.9	26.0–428.0	41.1 ± 33.2	13.0–158.0	62	45.9	<0.001
FPOM (mL)	10.9 ± 12.6	0.6–51.6	1.6 ± 1.9	0.1–8.0	62	44.3	<0.001
NW	9.3 ± 2.2	6.0–13.0	7.0 ± 1.6	4.0–10.0	62	22.3	<0.001
Protist abundances							
HNF (10^4 mL^{-1})	4.5 ± 6.1	0.9–35.8	1.4 ± 1.8	0.3–10.6	62	39.3	<0.001
Ciliates (10^2 mL^{-1})	4.3 ± 5.6	0.3–29.3	5.7 ± 10.3	0.0–42.0	62	3.3	0.07
Algae (10^4 mL^{-1})	10.1 ± 29.4	0.1–134.6	0.2 ± 0.4	0.01–1.8	62	18.0	<0.001
Testate amoebae (10^2 mL^{-1})	1.9 ± 5.3	0.01–29.0	0.8 ± 1.7	0.0–7.9	62	6.3	0.01
Naked amoebae (10^2 mL^{-1})	0.5 ± 0.8	0.0–2.5	1.3 ± 5.5	0.0–31.2	62	0.06	0.8
Protist richness (*S*)	13.2 ± 2.1	9.0–18.0	11.7 ± 2.2	7.0–16.0	62	7.3	0.009
Protist diversity (*H'*)	1.8 ± 0.6	0.3–2.8	2.2 ± 0.5	1.0–3.0	62	7.2	0.01

Counting other aquatic organisms

Bacteria and metazoa (i.e., rotifers and macroinvertebrates) were also counted and constituted a set of five "ecological" explanatory variables in subsequent analyses. For bacterial abundance, a 1 mL subsample was stained with SYBR Green (10^{-4} M) and analyzed through flow cytometry (Becton Dickinson FACS Calibur). Rotifers were counted after sedimentation in plankton chambers using the same method as described for ciliates and testate amoebae. Macroinvertebrates were sorted and preserved in 70% ethanol. They were identified to genus, species or morphospecies and then partitioned into "functional feeding groups" (FFGs) based on morphological and behavioral adaptations to food acquisition (Merritt & Cummins 1996). These functional groups were: collector–filterers, collector–gatherers, and predators. Because shredders were only found in a few samples and in low abundances, this FFG was omitted. FFG density was expressed as ind/mL.

Data analyses

Differences in protist diversity, the abundances of the different functional groups of protists, the amount of FPOM, water volume, the number of wells per plant, and the percentage of total incident radiation between Cf-*Aechmea* and Pg-*Aechmea* ($n = 31$ and $n = 32$, respectively) were tested using a one-way ANOVA. These analyses were conducted on log transformed data to meet the assumptions of normality and homogeneity.

Relationships between all environmental variables (including the five "ecological" explanatory variables), the bromeliads, and protist abundance data were examined using multivariate ordination. Protist abundances were $\log(n+1)$ transformed prior to analyses. An initial Detrended Correspondence Analysis (DCA) in CANOCO v4.5 showed that a linear model was most applicable because of low species turnover along axis 1 (gradient = 0.924, Lepš & Šmilauer 2003); thereafter, a Redundancy Analysis (RDA) was used to examine protist relationships with the 20 environmental variables. Forward selection was employed to test which of the environmental variables explained a significant ($P < 0.05$) proportion of the species variance. The significance of explanatory variables was tested against 500 Monte-Carlo permutations. Mann–Whitney tests were used to test significant differences in bromeliad distribution in the RDA according to the ant partner (Cf-*Aechmea* vs Pg-*Aechmea*) by using the coordinates of samples on the most significant axis.

Results

Pg-*Aechmea* were located in the sunniest part of the forest edge and thus, as was found in a previous study (Leroy et al. 2009), received significantly more light than Cf-*Aechmea* (Table 1). They contained lower water volumes, lower amounts of FPOM, and lower number of wells per plant than Cf-*Aechmea*. The abundances of HNF, algae and testate amoebae were significantly higher in Cf-*Aechmea* than in Pg-*Aechmea*. The mean abundances of ciliates and naked amoebae were not significantly different between the bromeliads (Table 1). Of the 25 protist morphospecies recorded, 22 were shared by both ant-gardens, while the three remaining were exclusive to Cf-*Aechmea* (See Appendix A: Table 1). The protist richness in Cf-*Aechmea* was higher than in

Pg-*Aechmea* but protist diversity, expressed by the Shannon (H') indice, was lower in Cf-*Aechmea* than in Pg-*Aechmea* (Table 1).

Axes 1 and 2 of the RDA accounted for 18.8% of the total species variance and 83.2% of the species–environment relationship. Eigenvalues for axes 1 and 2 were 0.15 and 0.04, respectively. Species–environment correlations were 0.840 for axis 1 and 0.759 for axis 2. Forward selection identified four variables as explaining a significant amount of the species variance (arrows in Fig. 1A): FPOM ($P = 0.002$), number of leaves ($P = 0.002$), incident radiation ($P = 0.01$), and number of epiphyte species ($P = 0.01$). None of the five ecological variables was significant in determining species distribution. RDA-scores for all of the variables tested are provided in the supplementary material (Appendix A: Table 2). FPOM accounted for the greatest proportion of the total canonical eigenvalues (12.5%; $F = 8.73$; $P = 0.002$). The scatterplot of the RDA allowed us to distinguish two main subsets along axis 1 when bromeliads were more specifically grouped according to the ant partner (Fig. 1A); i.e., the Pg-*Aechmea* area (left part of the scatterplot) and the Cf-*Aechmea* area (right area). Pg-*Aechmea* were less scattered than Cf-*Aechmea* in the scatterplot indicating a more homogenous protist community within individuals of Pg-*Aechmea*. Axis 1 displayed a gradient of phytotelm habitat conditions. There was a gradient of habitat complexity (total number of leaves) and amount of FPOM made available to the microorganisms from low (left side of the scatterplot) to high (right), and a gradient of incident radiation ranging from low (right) to high (left). Within these gradients, Pg-*Aechmea* individuals were found in sun-exposed areas and contained greater abundances of small-sized ciliates (Fig. 1B, see Appendix A: Table 1). These bromeliads exhibited low abundance for the majority of the algal taxa. In contrast to Pg-*Aechmea*, Cf-*Aechmea* showed lower abundances of large-sized ciliates but higher abundances of the majority of other protist taxa including HNF, algal and testate amoebae morphospecies (Fig. 1B, see Appendix A: Table 1). There was a highly significant difference in sample coordinates according to bromeliad species (left cluster = Pg-*Aechmea*, right cluster = Cf-*Aechmea*) along axis 1 ($P < 0.0001$). Finally, it appeared that ant-gardens associated with *C. femoratus* hold a higher diversity of epiphyte species than the ant-gardens associated with Pg-*Aechmea*.

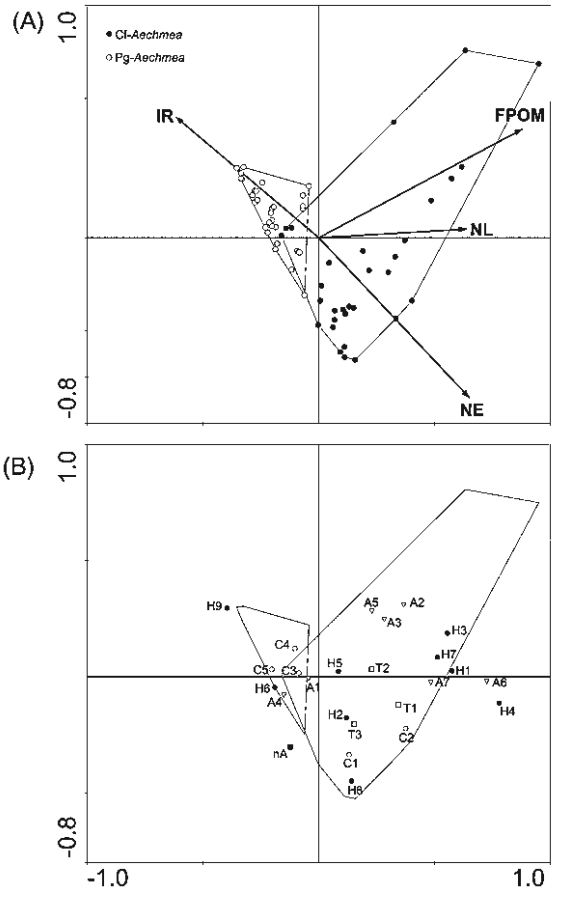

Fig. 1. Redundancy Analysis (RDA) biplots. (A) Bromeliads and environmental variables. Different symbols were used to indicate ant-garden species. Environmental variables are represented as vectors; directions show the gradients, the lengths of arrows represent the strengths of the variables in the ordination space. Only the variables explaining a significant ($P < 0.05$) proportion of the species variance are represented. Markers were used to identify the associated ant-species. Cf-*Aechmea* = bromeliads associated with *Camponotus femoratus*, Pg-*Aechmea* = bromeliads associated with *Pachycondyla goeldii*. (B) Distribution of protist taxa in the ordination space. The abbreviations for environmental variables are: NL = total number of leaves; NE = number of epiphyte species rooted on the ant-garden; IR = percentage of total incident radiation above the bromeliads; FPOM = amount of fine particulate organic matter (mL). The abbreviations for protist taxa are: H = heterotrophic nanoflagellates; C = ciliates: A = algae; T = testate amoebae; nA = naked amoebae. Details on the morphological characteristics and putative affiliation of protist taxa are provided in the supplementary material (Appendix A, Table 2).

Discussion

Although plant–insect interactions are pervasive in the tropics and elsewhere (McKey et al. 2005; Rico-Gray & Oliveira 2007), the influence of cross-scale interactions on microorganisms (i.e., how plant–insect interactions affect the microbial components of natural systems) has not yet been investigated. This is due to the fact that ecologists have primarily focused on the outcomes of predation by grazers/filter-feeders (including their cascading impacts on microbial food webs) (Sommer 2008) and/or resource availability (Hoekman, Winston, & Mitchell 2009), while the indirect impacts of non-trophic interactions between plants and/or animals (e.g., competition, mutualisms, chemical cues) on the entire community have been less often addressed (Adl & Gupta 2006). It is, however, well known that organisms may exert community-wide effects via physical changes to habitats with consequences for the structural

organization of the occupants and their functions (Jones, Lawton, & Shachak 1994). As dispersal agents for the tank-bromeliad *A. mertensii* in the Neotropics (Corbara, Dejean, & Orivel 1999), the ant-garden ants *C. femoratus* and *P. goeldii* determine the location of its seedlings, and, because these ant species have different nesting preferences as regards light intensity, they indirectly influence the phenotype of the plant (Leroy et al. 2009). *A. mertensii* reveals a significant plasticity in its vegetative traits, which influences both habitat characteristics (e.g., tank shape and size, number of leaves; Leroy et al. 2009, present study) and resource availability (e.g., water and litter) of the aquatic microecosystem. These changes induce an important shift in the structure of the protist community. Our results therefore demonstrate the decisive, indirect influence of the ant partner on the densities, diversity and functional structure of a complete microbial community via changes in habitat and abiotic conditions.

Among the 20 variables tested, we found four abiotic variables explaining the shift in protist richness between ant-garden bromeliads. The quantity of FPOM explained most of the variance in community structure. The partially shaded sites selected by *C. femoratus* permit the development of large tank-bromeliads that receive large amounts of organic matter from overhanging trees. Most of these bromeliads are a very favorable site for HNFs and testate amoebae which are known to consume both suspended and attached bacteria and organic matter. In addition, testate amoebae are frequently associated with organic surfaces and larger reservoirs which provide a more diverse range of microhabitats. In accordance with previous studies (Richardson 1999; Armbruster, Hutchinson, & Cotgreave 2002), the large amounts of organic matter found in these individuals sustain a higher diversity of macroinvertebrate FFGs (see Appendix A: Table 3). By fragmenting detrital organic matter particles, detritivorous invertebrates are known to enhance bacterial activity (Cummins & Klug 1979) and thus the predators of these bacteria, such as HNF and testate amoebae. Although we observed congruent patterns in the abundances of protists and macroinvertebrate FFGs, none of the biotic variables was significant in our analysis, suggesting that the putative positive impact of invertebrates on protists was likely minor.

Habitat complexity (number of leaves) also had a strong impact on the protist community by increasing the abundance and richness of the community. This result is consistent with numerous previous studies dealing with fauna from both non-ant-garden (Richardson 1999; Armbruster et al. 2002; Srivastava, Trzcinski, Richardson, & Gilbert 2008; Jabiol, Corbara, Dejean, & Céréghino 2009) and ant-garden bromeliads (Céréghino et al. 2010). In addition to larger quantities of detritus, larger numbers of sub-reservoirs per plant result in greater habitat complexity providing more micro-habitats and resources, reducing competition, and permit more species to coexist. As a result, protist richness was significantly higher in Cf-*Aechmea* individuals compared to Pg-*Aechmea* individuals. Our results also indicate that ant-gardens that had the highest epiphyte richness (4–5 species) were all associated with *C. femoratus*, and this richness positively affected the protist community. It should be noted that the abundances of large-sized protozoa (i.e., large-sized ciliate taxa and all of the testate amoebae taxa) increased with the number of epiphytes. These characteristics show that mature ant-gardens associated with *C. femoratus* are miniature spots of biodiversity containing a high level of richness of epiphytes and aquatic invertebrates (Céréghino et al. 2011), protists (this study), and almost certainly terrestrial arthropods (personal observations).

The percentage of total incident radiation received by the bromeliads strongly affects the morphology of the plant (Montero, Feruglio, & Barberis 2010). *A. mertensii* individuals associated with *P. goeldii* grew in sun-exposed areas and, as such, their rosettes had a small width and were amphora shaped (Leroy et al. 2009). The low quantities of FPOM and the lower complexity of these phytotelmata reduce the abundance and richness of most heterotrophic protists but also the abundance and richness of the algae. Thus, in contrast to our predictions, algae showed high abundances in the largest Cf-*Aechmea* which receive incident radiation two times lower than Pg-*Aechmea*. This result conflicts with the patterns of algal abundance in "non-ant-garden" bromeliads (Brouard et al. 2011) and suggests that the nutrients provided by the decomposition of organic matter, rather than light, are a key factor controlling algal growth in *A. mertensii*. The abundant bacteria and protozoa in the largest Cf-*Aechmea* might stimulate the rate of decomposition of organic matter, thus releasing more nutrients in forms available to algae (Ngai & Srivastava 2006). In contrast, the small amounts of organic matter and the resulting low abundances of microbial decomposers and their predators in Pg-*Aechmea* revealed a nutrient-limited microcosm likely to restrict algal growth as light is not a limiting factor for photosynthesis in this case. Also, it is likely that the trichome-coated Pg-*Aechmea* leaves outcompete algae for nutrient uptake in water- and nutrient-stressed conditions. The predominance of ciliate taxa within the protist community of Pg-*Aechmea* reinforced the idea of a low level of productivity in these phytotelmata. Indeed, owing to their higher volume-specific clearance rates in comparison to other protozoa, ciliates are undoubtedly better adapted to consuming suspended prey in a diluted environment such as the phytotelmata of Pg-*Aechmea*.

Because ant–plant interactions determine the light-exposure conditions, the structure of the container habitat, and the amount of detritus at the base of the aquatic food web in the bromeliad *A. mertensii*, functional shifts (mostly abundance patterns) occur as the protist community structure changes from one ant partner to the other. This study therefore underscores how one level of interspecific interaction (an ant–bromeliad mutualism) can affect another level of interaction (the bromeliad food web), thereby influencing the functional groups of protists and microbial food web structure. Because *A. mertensii* is common and has never been found growing outside ant-gardens in the

Neotropics, the role of ants in creating microcosms and maintaining aquatic microbial diversity along forest edges is critical.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.baae.2012.10.002.

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